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OBSERVATIONS ON THE INHERITANCE OF SEX IN SCIARA OCELLARIS
(DIPTERA)

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This paper deals with the problem of the inheritance of sex in the fungus fly *Sciara ocellaris*, Comstock. It has been previously shown by Metz and his associates (see Metz, 1929, for a summary of the work) that in *S. coprophila* the females regularly yield "unisexual progenies." That is, the offspring of one female are all or nearly all of one sex, the term "progeny" being used by Metz and in this paper to designate the offspring of a single female. In another species, *S. reynoldsi*, the females regularly yield "bisexual progenies." In this case among the offspring of one female there may be both males and females in various ratios, usually not 1:1. In *S. ocellaris* it was found by them that some females yield unisexual and some bisexual progenies.

In Sciara the sex of the individual is not determined by the kinds of chromosomes in the zygote but by the results of the chromosome elimination which occurs during the early development of the embryo. Cytological and genetic investigations on Sciara by Metz and his co-workers have shown that there are two X chromosomes in the germ line of each sex. Oogenesis is typical and each ovum has one X chromosome. Spermatogenesis is atypical. In the first spermatocyte division a unipolar spindle is formed and the chromosomes of maternal origin go to the pole while those of paternal origin go away from it and are budded off and disintegrate. In the second spermatocyte division, which is bipolar, both halves of the X chromosome go together, precociously, to one pole. The chromosomes which go to the other pole are budded off and lost. The resulting cell is haploid except for two X chromosomes, which are genetically equal, the products of the division of one. Thus all male gametes contain two X chromosomes and all the gametes of any individual male are genetically alike, containing the maternal complement of genes (Metz 1926, 1928, 1929). The fertilized egg is then trisomic, containing three X chromosomes.

The significance of three X chromosomes in the fertilized egg is not clear, for during cleavage the number is reduced to one in the soma of the male, to two in the soma of the female, and to two in the germ-line of both (DuBois 1933, Berry 1939). The eliminated chromosomes are normally paternal in origin. At least one of the genetically-equal paternal X chromosomes is normally eliminated from all cells before the embryo develops. The sex of the individual is determined by the number of X chromosomes remaining in the soma after elimination has taken place. If one remains the individual becomes a male, if two, a female.

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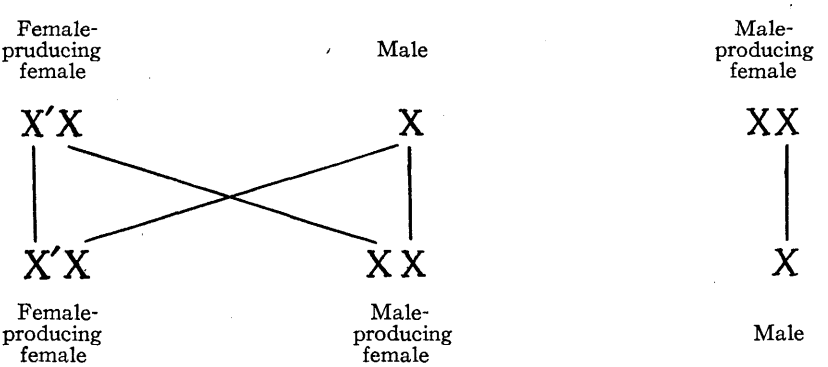
A cytological difference between *S. coprophila* and *S. ocellaris* is the presence of "limited chromosomes" in *S. coprophila* and their absence in *S. ocellaris*. In *S. coprophila* these are limited to the germ-line of both sexes. It has been shown that the limited chromosomes are not sex-determiners (Metz and Ullian, 1929) and they will not be considered further here. Otherwise the two species seem to be alike cytologically.

The X chromosomes have been found to be of two kinds in *S. coprophila* (Metz and Schmuck, 1929, Metz and Smith, 1931) and have been designated X and X' (X prime). The X' chromosome differs from the X in possessing a dominant "prime factor," which is not necessarily a single gene but may be two or more apparently completely linked genes or a segment of the chromosome. No crossing-over has been observed between the X and X'. It has been demonstrated that females carrying the X' chromosome are female-producers. That is, they yield progenies which are entirely of females or nearly so. Females homozygous for the X chromosomes are male-producers. Males from male progenies do not have the X' chromosome. Hence female-producers mated to males of male progenies are expected to yield female-producing and male-producing daughters in a ratio essentially 1:1. The X and X' chromosomes have both been genetically marked. Four recessives have been found on the X and a dominant on the X'.

This may be more easily seen with the aid of a diagram (Fig. 1). The male may be represented by X since it effectively transmits only one.



A female-producing female is heterozygous for the prime factor, and so may be considered digametic. A male-producing female does not have this factor on either of its X chromosomes and so is monogametic. As previously explained, the males get their somatic X from the female parent. Although one of the two X chromosomes in the germ-line of the male is paternal, it is not transmitted.

Although the presence or absence of a prime factor in the female parent determines the sex of the progeny as a whole it does not determine the sex of the individual offspring, for "exceptional" individuals occur. That is, a few females are found in some of the male progenies and a few males occur in some of the female progenies. As expected, the exceptional females are male-producers for they are without the prime factor. It will be presently shown that this condition does not exist in *S. ocellaris*. Here both unisexual and bisexual progenies occur. No prime factor is found and the evidence points toward the conclusion that a number of sex-determining factors affecting the sex ratio are present, some pulling in one direction and some in the other.

MATERIALS

The original flies obtained for this work came from cultures 1688 and 1692 of the laboratory of C. W. Metz. They are from a stock (1255) originally secured

by J. P. Reynolds in Alabama. The yellow flies used in the crosses were from the mutant strain discovered by E. G. Lawrence in this laboratory. The genes for yellow body and curled wing are sex-linked recessives and both known to be consistent in expression.

EXPERIMENTAL RESULTS

Tests for the prime factor.

As previously explained, females of *S. coprophila* typically produce unisexual progenies, female or male, depending on whether the female parent is genetically X'X or XX. No crossing over has been found to occur between the X' and X chromosomes and only about .5% has been observed between the two genes on the X. The X' and X chromosomes are both genetically marked in this species. Hundreds of tests carried on over a period of years and all giving consistent results (Metz and Schmuck, 1929a, Metz and Smith, 1931) have shown that females carrying the X' chromosomes are female-producers and those homozygous for the X are male-producers.

TABLE I

SETS OF SIBS WHICH PRODUCED MALE AND FEMALE PROGENIES IN A RATIO HIGHLY SIGNIFICANT IN THEIR VARIANCE FROM A 1:1 RATIO. TEN SETS OUT OF 54 IN THE UNISEXUAL STRAIN AND EIGHT OUT OF 25 IN THE BISEXUAL

	Female Progenies	Male Progenies	Probability
Unisexual strain	9	26	.0092
	0	8	.0078
	13	2	.0074
	13	2	.0074
	35	14	.0040
	9	0	.0040
	0	9	.0040
	42	18	.0024
	1	13	.0020
	45	5	.0000
Bisexual Strain	19	5	.0064
	2	14	.0040
	13	1	.0020
	10	32	.0008
	31	6	.0000
	1	21	.0000
	0	15	.0000
	39	0	.0000

When similar tests are applied to *S. ocellaris* the results obtained are different. There are two sex-linked genes in this species, yellow and curled, both recessive. Recombination between them is about 13%. The yellow gene has been used in the present work.

Yellow females may be either female-producers or male-producers. In this they differ from the females of *S. coprophila*, where a genetic marker designates either an X or X' chromosomes and therefore either a male-producer or a female-producer. Recessives on the X' of *S. coprophila* would not ordinarily show, as females are not X'X'. It appears, therefore, that if a prime factor exists in *S. ocellaris* it is not completely linked with the yellow locus.

On the basis of conditions found in *S. coprophila*, as already outlined, it would be expected that in *S. ocellaris* half the females in female progenies would be female producers (X'X) and the other half male-producers (XX). This assumes

that the male parents are derived from male progenies and hence carry only X and not X'. Exceptional males from female progenies are not used because of the possibility of some of them carrying an X' chromosome. This expectation is not realized in *S. ocellaris*. Among sisters in this species the ratio of female-producers to male-producers is frequently far from 1:1. Out of 54 sets of sisters from unisexual stock in which eight or more sisters were tested in each set, the divergence from a 1:1 ratio was highly significant in ten sets. That is, in these cases the probability of getting the ratio obtained by chance when a 1:1 ratio was expected was less than one chance in a hundred. Out of a sample of 54 it would not be very uncommon to get by chance a case which would by this definition be highly significant, but to get ten out of 54 is not within the realm of probability. Similarly in 25 such sets in bisexual stock there were eight cases in which the divergence from a 1:1 ratio was highly significant. The counts and respective probabilities are given in Table I. It is to be noted from the table that in some cases the probability is far below .01 and that in some others the size of the sample is rather small and might have produced a still more significant variation from the 1:1 ratio had the sample been larger. These sets of sisters were not among those tested for the prime factor with genetic markers nor were they from the female-producing line mentioned later.

TABLE II

FEMALE-PRODUCERS AND MALE-PRODUCERS IN PROGENIES WHERE AN EFFORT WAS MADE TO MATE ALL SISTERS

Females in Progeny	Female-Producers	Male-Producers	Probability on Basis of 1:1	Percent of Females Giving Progenies
77	25	28	.7948	69
82	34	30	.7082	68
106	33	38	.6350	67
56	26	20	.4616	82
99	47	38	.3858	86
60	30	20	.2024	83
88	47	31	.0888	89
69	42	18	.0024	87
71	45	5	.0000	70

In bisexual progenies there is a tendency for males to hatch earlier than females. If, similarly, there is a tendency for one type of female to hatch before the other, a skew ratio might be obtained when none in fact existed provided females hatching first were mated and those hatching later discarded. This, however, appears not to be the case for the following reasons: (1) As will be seen in Table I, among the unisexual lines which gave highly significant skew ratios six were predominantly female and four predominantly male, and in the bisexual line four were female and four male. If there had been an unintentional selection of females hatching early or late the skew ratios would be expected to be predominantly all of the same sex. (2) When the date of hatching of each female was recorded the results indicated no tendency for one type of females to hatch earlier than the other. (3) When all the females of a progeny were mated and a skew ratio obtained there could be no selection except selective mortality or sterility. Table II shows results where an effort was made to obtain offspring from each female in a progeny, with better than 60% yield. In two progenies out of nine a highly significant skew ratio occurred.

From the evidence presented it is concluded that ratios differing from 1:1 as to female-producers and male-producers do occur in female progenies in this species.

Test for the prime factor with genetic markers.

The recessive sex-linked gene yellow in *S. ocellaris* may be used to test directly whether there is a prime factor on an X chromosome determining the sex of progeny as there is in *S. coprophila.*

If a female progeny is obtained from a homozygous wild-type female by a yellow male, the female offspring are wild-type in color and heterozygous for yellow. If a prime factor operates as in *S. coprophila*, it should be derived in this case from the wild-type mother. If the F_1 females are mated to yellow males some of their daughters will be wild-type and some yellow, and except for cases involving crossing-over the wild-type daughters should be female-producers and the yellow ones male-producers. This is represented diagrammatically in Fig. 2 below, where + stands for wild-type and y for yellow. A male is represented by only one gene as a male effectively transmits only one X chromosome. Fig. 3 shows the crosses which would yield the opposite condition, in which wild-type daughters should be male-producers and yellow daughters female-producers.

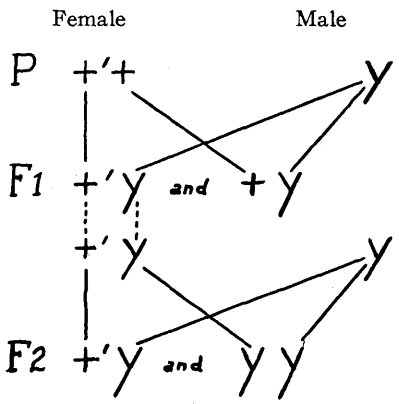


Fig. 2

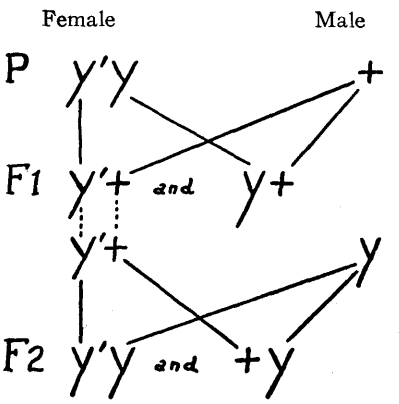


Fig. 3

Crosses of the first type yielded from three progenies, 73 wild-type F_2 females, all expected to be female-producers. Actually, 38 proved to be female-producers and 35 to be male-producers. Eighty-two of their yellow sisters, expected to be male-producers, included instead, 47 female-producers and 35 male-producers. Neither 38 to 35 nor 47 to 35 is significantly different from a 1:1 ratio, and from these figures to postulate a prime factor one would need to assume 50% crossing over or non-linkage.

In the second cross 173 females, of which 90 were wild-type and 83 yellow, were secured from five progenies. They produced progenies as follows: Of the 90 wild-type females expected to be male-producers, 60 were female-producers and 30 male-producers, which is a highly significant variation from the 1:1 ratio. Of their 83 yellow sisters, which were expected to be female-producers, 41 were female-producers and 42 were male-producers, a 1:1 ratio. Yellow females of another progeny, of which no wild-type sibs were saved, turned out to be 3 female-producers and 17 male-producers. This is a highly significant variation from the 1:1 ratio and in the direction opposite from the expected.

These data make it clear that there is no prime factor on the X chromosome acting as a unit as in *S. coprophila* and linked either completely or incompletely with yellow, nor is there a unit autosomal prime factor showing independent assortment with yellow. These results are given in Table III.

TABLE III

SHOWING THAT A "PRIME FACTOR" OPERATING AS IN *S. coprophila* IS NOT ACTING IN THIS SPECIES. IF IT WERE, IN THE FIRST CROSS THE WILD-TYPE SIBS WOULD ALL BE FEMALE-PRODUCERS AND THE YELLOW ONES ALL MALE-PRODUCERS, WHILE IN THE SECOND CROSS THE RESULT WOULD BE JUST THE OPPOSITE

Parent Culture	WILD-TYPE SISTERS		YELLOW SISTERS	
	Female-Producers	Male-Producers	Female-Producers	Male-Producers
First cross (figure one)				
AKI	6	6	17	4
BDZ	19	17	11	11
BEA	13	12	19	20
Total	38	35	47	35
Second cross (figure two)				
FY	4	2	7	2
HX	4	5	3	5
HY	7	0	3	7
AUB	20	10	9	10
AUD	25	13	19	18
Total	60	30	41	42
GCM	.....	.....	3	17

Evidence from a female-producing line.

It was found by Metz and Schmuck (1929b) that female-producing females of *S. coprophila* could have X^1 sons, which when mated to female-producing females would yield some X^1X^1 females. The daughters of such females are all female-producers and their exceptional sons carry and transmit the X^1 chromosome. By continually mating females to their exceptional brothers, only female-producers should be obtained, no matter how many generations this be carried on.

A female-producing line of *S. ocellaris* was obtained from a female caught in one of the government greenhouses in Washington, D. C. For three inbred generations all females tested were female-producers. As there were 129 of these, with an average of 75 offspring per female, the results cannot possibly be attributed to chance.

Lest a misunderstanding arise it should be explained that in the inbred female-producing line not all females tested were mated to their brothers, as there were not sufficient exceptional males to do this. The exceptional males were mated to as many of their sisters as possible to produce inbred lines and the rest of the females were mated to other males to ascertain whether or not they were female-producers. It has been shown by Metz that in *S. coprophila* the male parent does not affect the sex of the immediate progeny. It may be noted that if there were any effect it would probably tend to prevent rather than bring about the result here obtained, since the other males were not from female-producing lines.

Thus if there were a single prime factor operating, all future inbreeding should yield only female progenies. This was not the case. By selecting females from

progenies in which there were fewer exceptional males the female strain was maintained, while at the same time selecting females from progenies in which there were more exceptional males a large proportion of male progenies was obtained after a few generations. These results are summarized in Table IV. Apparently male-producers were obtained by chance combinations of factors for male-produc-

TABLE IV

RESULTS OF SELECTION IN A MALE DIRECTION IN A FEMALE-PRODUCING LINE, BEGINNING AT THE SEVENTH GENERATION. EACH NUMBER REPRESENTS THAT MANY PROGENIES. THEY ARE DIVIDED INTO GROUPS CORRESPONDING TO THE PER CENT OF THE MINORITY SEX IN THE PROGENY

	FEMALE PROGENIES					MALE PROGENIES				
	0–9	10–19	20–29	30–39	40–49	40–49	30–39	20–29	10–19	9–0
First generation	1	0	0	0	0	0	0	0	0	0
Second generation	4	6	4	3	4	3	2	4	4	2
Third generation	12	25	17	13	7	10	3	2	3	0
Fourth generation	46	10	2	0	0	0	0	0	0	0
Fifth generation	14	10	3	0	0	0	0	0	0	0
Sixth generation	38	5	0	0	0	0	0	0	0	0
Seventh generation	55	24	12	5	2	1	1	0	0	0
Female line	9	2	1	1	0	0	0	0	0	0
Male line	10	5	2	0	1	0	1	0	0	0
Eighth generation										
Female line	8	3	0	0	0	0	0	0	0	0
Male line	1	4	2	0	0	6	2	3	4	4
Ninth generation										
Female line	28	6	0	0	1	0	0	0	0	0
Male line	7	1	2	1	4	2	0	2	2	7
Tenth generation										
Female line	46	22	6	1	2	0	0	0	0	0
Male line	0	1	1	1	1	8	6	8	7	12
Three sets tested for the prime factor. See text and figure 4.										
Wild-type females	1	3	8	7	5	4	1	0	0	7
	1	1	6	8	5	1	3	0	0	7
	0	3	4	7	3	1	0	1	0	2
Total for wild-type females	2	7	18	22	13	6	4	1	0	16
Yellow females	1	1	0	2	1	3	0	2	0	22
	0	0	3	2	2	2	1	0	0	21
	0	1	0	2	2	0	0	0	0	6
Total for yellow females	1	2	3	6	5	5	1	2	0	49
Total for wild-type and yellow females	3	9	21	28	18	11	5	3	0	65

tion or lack of female-production. It should be possible to obtain a stable female-producing strain by continued selection until flies are obtained which are homozygous for the factors which make for female-production.

At the same time a male-producing line was obtained from the same original female from which the female-producing line mentioned above was obtained. This line was essentially bisexual and so differed from a male-producing line in *S. coprophila*, which could only be obtained by mating exceptional females (which

are without the prime factor) to their brothers. Out of 105 females tested during six generations, all but three produced more males than females. Of these three, two produced only one more female than males and the other produced two more females than males.

The female-producing females were also outcrossed to yellow so as to mark the X chromosome. If a single prime factor were located on this chromosome without crossing over, the result should be as follows (Fig. 4) when the first generation females are backcrossed to yellow.

The wild-type females are all expected to be female-producers and the yellow females are expected to be male-producers. The results may be seen in Table IV, a number of the progenies were bisexual, especially from the wild-type females.

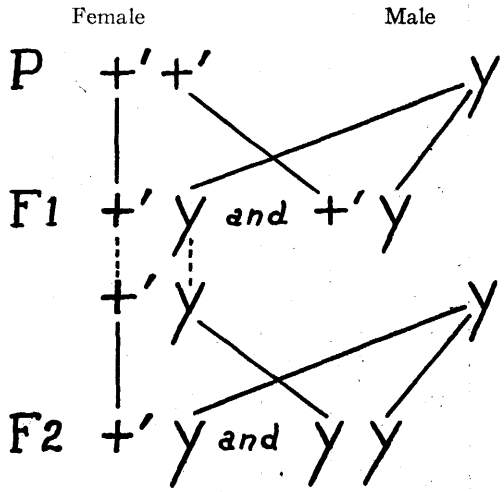


Fig. 4

The line dividing progenies between the categories predominantly male and predominantly female appears to divide the progenies arbitrarily and the natural division falls between the unisexual male progenies on the one hand and all the others. This is even more striking than the table indicates, for of the 65 unisexual male progenies two had one exceptional female each and all the others, with an average of 102 flies each, were all males with no exceptional females.

If the unisexual male progenies are considered as the expected male progenies and all the others as the expected female progenies, the results for the wild-type females are, 75 non-crossovers and 16 crossovers, and for the yellow females, 49 non-crossovers and 25 crossovers. If these numbers are set in a two-by-two table it is found that chi-square is 5.37, which is significant, though not highly significant. The results, therefore, do not preclude the possibility that a factor linked with the locus of yellow and crossing over with it is operating here and determining a bisexual and unisexual condition.

Bisexual females from the strain just considered seem to have three X chromosomes in their germ-line instead of two, a condition observed by Reynolds (1938) in a bisexual strain of *S. coprophila*. He found that some of the ova get two X chromosomes and some get one, in oogenesis. The former become females and the latter males. This does not involve the X' chromosome and in returning to the normal condition the females all were male-producers.

The evidence that the three-X condition existed in this line is as follows: (1) Wild-type and yellow females were produced in some crosses between wild-type females and wild-type males. Ordinarily yellow females are never found in progenies resulting from such crosses because a wild-type male transmits only the X chromosome bearing the wild-type gene. This is so because in the developing male the two X chromosomes of paternal origin in the trisomic zygote are eliminated in the somatic tissue and one is eliminated in the germ-line. Later, when germ cells are formed, all the chromosomes of paternal origin are lost in the first

TABLE V

SHOWING THE DISPERSION ON A MALENESS-FEMALENESS SCALE OF THE PROGENIES OF FOUR GROUPS OF CLOSELY RELATED FEMALES. IT IS TO BE NOTICED THAT THE PATTERN IS SIMILAR FOR FEMALE-PRODUCERS AND SIMILAR FOR MALE-PRODUCERS IN EACH GROUP, REGARDLESS OF THE SEX-LINKED PHENOTYPE, BUT DIFFERENT WHEN FEMALE-PRODUCERS ARE COMPARED WITH MALE-PRODUCERS. THE NUMBERS STAND FOR PROGENIES, NOT INDIVIDUAL FLIES

	INDIVIDUALS OF MINORITY SEX PER 100 OF MAJORITY SEX										
	0	1–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99
I. Wild-type female-producers	0	2	3	6	15	9	12	4	5	6	0
Yellow female-producers	0	2	1	1	2	2	2	3	1	1	2
Wild-type male-producers	16	0	0	0	1	0	3	2	1	3	1
Yellow male-producers	47	2	0	0	2	0	0	1	2	3	0
II. Wild-type female-producers	0	9	6	2	2	0	0	0	0	0	1
Yellow female-producers	0	4	2	2	1	0	0	0	0	0	1
Wild-type male-producers	16	1	0	0	0	0	0	0	0	0	1
Yellow male-producers	10	1	0	0	0	0	0	0	0	0	0
III. Wild-type female-producers	5	8	0	0	0	0	0	0	0	0	0
Yellow female-producers	6	13	0	0	0	1	0	0	0	0	0
Wild-type male-producers	0	3	0	2	6	4	1	0	1	0	0
Yellow male-producers	1	6	6	4	2	2	0	0	0	0	0
IV. Wild-type female-producers	4	8	2	1	1	1	1	0	0	0	0
Yellow female-producers	4	11	0	1	0	1	1	0	0	1	0
Wild-type male-producers	12	2	1	1	0	0	0	0	0	0	0
Yellow male-producers	9	3	0	0	0	0	0	0	0	0	0

spermatocyte division, as previously explained. (2) Yellow females from the cross just described mated to yellow males yielded wild-type and yellow males and females. Ordinarily in crosses of yellow by yellow the offspring are all yellow, as any fly which could transmit the wild-type gene would manifest it phenotypically. (3) That such females have in their germ-line three X chromosomes rather than being heterozygous and having two is further indicated by the ratio of the offspring. Heterozygous females mated to yellow males produce wild-type and yellow offspring in a ratio close to 1:1. But if a female has two X chromosomes bearing yellow and one bearing wild-type, it is to be expected that twice as many female offspring will be wild-type as yellow and twice as many male offspring will be yellow as wild-type. In general this proportion was realized in these cases but

sometimes the ratio was still farther from 1:1 than expected, indicating a tendency for the yellow chromosomes to segregate more frequently than would be expected by chance.

It may be seen from the entries in Table IV under the sets tested for the prime factor, and still more clearly in Table V, that the distribution pattern is similar for the wild-type and yellow female-producers and similar for the wild-type and

TABLE VI

THE PROGENIES OF SISTER FLIES, GROUPED TO SHOW THEIR DISTRIBUTION ON A MALENESS-FEMALENESS SCALE. THE NUMBERS REPRESENT PROGENIES, NOT INDIVIDUALS. THEY ARE ARRANGED IN COLUMNS ACCORDING TO THE PER CENT OF THE MINORITY SEX IN EACH PROGENY. EACH ROW REPRESENTS A SET OF PROGENIES FROM SISTERS, AND THE SETS ARE ARRANGED IN ORDER, BEGINNING WITH THOSE HAVING THE MOST FEMALE PROGENIES

PERCENT FEMALES					PERCENT MALES					TOTALS	
0-9	10-19	20-29	30-39	40-49	40-49	30-39	20-29	10-19	0-9	♀ Progenies	♂ Progenies
11	13	9	2	4	0	0	0	0	0	39	0
5	6	5	7	8	3	2	0	1	0	31	6
0	6	1	4	8	1	2	1	0	1	19	5
1	1	2	4	3	3	1	0	1	0	11	5
3	4	3	1	2	5	0	0	1	0	13	6
0	1	5	6	5	3	4	1	0	0	16	8
0	3	7	8	7	6	5	2	0	0	25	13
0	0	4	5	3	4	0	4	0	0	12	8
2	2	3	2	3	5	2	1	2	0	12	10
0	0	1	6	0	1	1	3	1	0	7	6
0	0	4	6	2	4	4	2	1	0	12	11
0	3	0	0	4	2	3	1	2	0	7	8
1	1	4	7	7	6	7	6	4	1	20	24
0	1	1	2	5	4	3	4	3	0	9	14
0	1	2	1	1	3	1	2	4	2	5	12
0	1	0	2	2	5	5	1	2	0	5	13
0	0	2	5	3	10	4	9	6	3	10	32
0	0	0	3	0	0	1	3	5	1	3	10
0	0	0	1	1	1	0	7	6	0	2	14
0	0	0	0	1	0	2	3	6	2	1	13
0	0	0	1	0	4	8	7	1	1	1	21
0	0	0	0	0	2	3	4	4	2	0	15
Totals of the three groups respectively.											
19	33	33	38	41	26	13	9	2	1	166	51
3	6	12	21	16	18	17	13	11	1	58	59
0	3	5	15	13	29	27	40	37	11	36	144

yellow male-producers, but is different in the female-producers as compared with the male-producers. Each number represents that many progenies and in Table V they are arranged in columns by the number of individuals of the minority sex in each progeny per hundred of the majority sex. The first four rows are the same as the totals for the three sets tested for the prime factor entered in Table IV. This indicates some factor independent of the X chromosome but associated with the determination of sex of progeny which determines, within certain limits, whether the progeny is unisexual or bisexual.

It will be seen in Table IV under the three sets tested for the prime factor and the totals given there that the distribution is bimodal. In unisexual lines the distribution is strictly bimodal, with the female progenies at one end of the distribution and the male progenies at the other. In some bisexual sets the distribution of the progenies of sisters has a single mode. The number of progenies with more females and the number with more males frequently are not in a 1:1 ratio. This is shown in Table VI, where twenty-two closely related sets of progenies are tabulated. It will be seen that the progenies of sisters tend to group together when arranged on a scale of femaleness and maleness and when one sex predominates there is a tendency for more progenies of that sex to be unisexual than for progenies of the other sex.

Some crosses have been made between unisexual and bisexual lines and the first generation females tested to find out what kind of progenies they yield, but much remains to be done. As may be seen in Table VI, there are frequently some unisexual progenies in a bisexual line and the distribution of the progenies forms a curve with a mode somewhere near the center and with the ends tapering off. Females from unisexual progenies derived from a bisexual line may yield bisexual progenies, though a continued selection for unisexual progenies would probably after a time yield a stable unisexual line.

TABLE VII

THE RESULTS OF SOME CROSSES BETWEEN UNISEXUAL AND BISEXUAL STRAINS

Parentage of First Generation	Number of First Gen. ♀ ♀ Tested	Nature of Second Gen. Progenies	Exceptionals per 100 of Others
♀ unisexual, ♂ unisexual..	86	Unisexual.....	1.2
♀ unisexual, ♂ bisexual...	69	Unisexual.....	1.3
♀ unisexual, ♂ bisexual...	53	Some unisexual, some bisexual.....	15.7
♀ unisexual, ♂ bisexual...	91	Bisexual.....	58.3
♀ bisexual, ♂ unisexual...	44	Some unisexual, some bisexual.....	9.5
♀ bisexual, ♂ bisexual....	102	Some unisexual, some bisexual.....	32.0

In Table VII is presented the results of several crosses. The unisexual progenies mentioned here are all from stable unisexual lines. The variation in results, the fact that unisexual progenies from a bisexual source are not stable, and the grouping of the progenies of sisters about a mode which is not the same for different sets of sisters, indicate the presence of more than one factor and its allele determining the sex ratio of the progenies.

DISCUSSION

There are three different problems in the determination of sex in Sciara: (1) the determination of the sex of the individual, (2) the determination of the sex of progenies, and (3) the determination of the sex of groups of closely related progenies, such as the progenies of sisters.

The determination of the sex of individuals by the elimination of chromosomes has already been discussed at length. Gynanders are not uncommon in *S. ocellaris*. The division is usually roughly with the longitudinal axis of the body. When the female parts are wild type and the male parts are yellow they may be readily distinguished and much variation in pattern is observed among individuals.

To discover whether a sex-linked "prime factor" similar to that in *S. coprophila* occurs in *S. ocellaris*, three types of crosses were made, each of which would reveal

the presence of such a factor. The results expected on the assumption of this factor were not realized in any of the crosses. In some cases the number of female-producers was essentially the same as the number of male-producers among sisters which were all expected to be male-producers, and vice versa. In some cases skew ratios were obtained, which occurred in the direction opposite to the expected as well as in the expected.

In keeping stock cultures of *S. ocellaris* one of the difficulties encountered is that a line may at any time "go male" or "go female" so that it becomes difficult to provide for the next generation. If a single factor were operating to determine the sex of progeny, male and female progenies should be produced each generation in essentially equal numbers. Also a female-producing line would remain such as long as inbred with exceptional males instead of becoming male after a few generations of selection.

Skew ratios sometimes occur in the inheritance of the X chromosome from heterozygous females, but this cannot be related to the skew ratios of sex of progeny, for it is far less frequent and a much larger sized sample is necessary to detect it.

There may be a marked similarity in the distribution on a maleness-femaleness scale among progenies of the female-producers of both phenotypes and among the male-producers of both phenotypes, while no such similarity is found when the female-producers are compared with the male-producers (Table V). From this it appears that a factor or factors determining the distribution of progenies is associated with the determination of sex of progeny, and seems to be autosomal since it is independent of the sex-linked phenotypes.

In other cases sisters of one phenotype produced male and female progenies in a ratio significantly skew while their sisters yielded male and female progenies in a ratio of 1:1 (Table III, AKI, AUB, AUD). In each case the size of the sample was not great enough to show the two sets of data significantly different from each other in a two-by-two table. If the difference is real, its cause seems to be a sex-linked factor.

Where the sex of progenies is distributed along a scale from unisexual to bisexual, the dividing point between these two groups becomes arbitrary. In such cases progenies were called bisexual if there were ten or more individuals of the minority sex per hundred of the majority sex, for in unisexual strains the ratio of exceptionals does not ordinarily exceed this.

The age of the female parent appears to make no difference in the sex of progeny, as no significant difference was observed among progenies of the second generation from females mated when less than eight hours old and females mated when over one hundred twenty-three hours old. These lines were closely related, as the females tested were sisters and they were mated to brothers.

External environment also seems to be eliminated as a factor in these results. No tests were made to ascertain this, but cultures were kept together on the same table and frequently some groups of sisters would be yielding bisexual progenies at the same time that others were yielding unisexual progenies, and some groups of progenies would be predominantly male at the same time that others were predominantly female.

From the facts obtained in the present study it is concluded that sex of progeny in *S. ocellaris* is determined by a number of factors, predominantly autosomal, though some may be sex-linked. It is certain that a determiner acting as a unit like the prime factor in *S. coprophila* does not exist in *S. ocellaris*, at least in the strains thus far studied. To ascertain more definitely where these determiners are it is desirable or necessary to have a number of autosomal markers and viable deletions.

Since no crossing over has been observed in *S. coprophila* between the X and X' chromosomes, though there are two markers on each, there is a possibility that the prime factor actually is multiple in this species also and spread over the X' chromosome. A transition from one species to the other, accompanied by translocations, could cause such a multiple factor on one chromosome to become scattered on other chromosomes. On this hypothesis, *S. coprophila* would be the older species. In the contrary case, a concentration of factors on one chromosome by translocations from others would be implied, which seems less likely.

SUMMARY

1. *Sciara ocellaris* produces both unisexual and bisexual progenies, in contrast to *S. coprophila*, which typically produces only unisexual progenies. In *S. ocellaris* some unisexual strains are stable. Apparently all bisexual strains are unstable, for if a number of sisters are tested some yield unisexual progenies and some bisexual. The distinction between these two types of progenies is not always clear, for there may be a complete series from unisexual progenies to those in which males and females are present in a ratio of 1:1. Females of unisexual progenies from a bisexual source may yield bisexual progenies.

2. From the results of three kinds of crosses it is shown that the sex of progeny is not determined by the presence or absence of a single sex-linked "prime factor," as it is in *S. coprophila*. In no case were the results expected on this assumption realized.

3. It is shown that no single autosomal "prime factor" exists in this species. By selecting less strongly female progenies of a female-producing line, male progenies were obtained after several generations, while selecting for the more strongly female progenies continued a female-producing strain. On the assumption of a single autosomal factor, the strain would be homozygous and no amount of selection would affect it.

4. Evidence that a sex-linked factor or factors may be operating is given in that male and female progenies of sisters of one sex-linked phenotype may occur in a skew ratio while the progenies of their sisters of the other phenotype occur in a 1:1 ratio.

5. Also there is evidence of autosomal factors, since the distribution on a maleness-femaleness scale may be strikingly similar for progenies of female-producing and male-producing sisters of both phenotypes, but dissimilar (not mirror-image distributions) when the male-producing and female-producing sisters are compared.

6. Female-producers heterozygous for a single "prime factor" should yield female-producing and male-producing females in essentially equal numbers. However, a number of ratios occur which differ significantly from a 1:1 ratio, and seem to be free of any accidental selection.

7. These results do not appear to have been influenced by external or internal environment.

LITERATURE CITED

Berry, R. O. 1939. Observations on chromosome elimination in the germ cells of *Sciara ocellaris*. Proc. Nat. Acad. Sci. 25: 125–127.

Du Bois, A. M. 1933. Chromosome behavior during cleavage in the eggs of *Sciara coprophila* (Diptera) in relation to the problem of sex determination. Zeit. f. Zellf. u. mik. Anat. 19: 595–614.

Metz, C. W. 1926. Genetic evidence of a selective segregation of chromosomes in *Sciara* (Diptera). Proc. Nat. Acad. Sci. 12: 690–692.

1928. Genetic evidence of selective segregation of chromosomes in a second species of *Sciara* (Diptera). Proc. Nat. Acad. Sci. 14: 140–141.

1929. Selective segregation of chromosomes in males of a third species of *Sciara*. Proc. Nat. Acad. Sci. 15: 339–343.

1938. Chromosome behavior inheritance, and sex determination in *Sciara*. Amer. Nat. 72: 485–580.

Metz, C. W., and **M. L. Schmuck.** 1929a. Unisexual progenies and the sex chromosome mechanism in *Sciara*. Proc. Nat. Acad. Sci. 15: 863–866.

1929b. Further studies on the chromosome mechanism responsible for unisexual progenies in *Sciara*. Tests of "exceptional" males. Proc. Nat. Acad. Sci. 15: 867–870.

Metz, C. W., and **H. B. Smith.** 1931. Further observations on the nature of the X-prime (X′) chromosome in *Sciara*. Proc. Nat. Acad. Sci. 17: 195–198.

Metz, C. W., and **S. S. Ullian.**..1929.. Genetic identification of the sex chromosome in *Sciara* (Diptera). Proc. Nat. Acad. Sci. 15: 82–85.

Reynolds, J. P. 1938. Sex determination in a "bisexual" strain of *Sciara coprophila*. Gen. 23: 203–220.